

## ANATOMICAL AND MORPHOLOGICAL CHARACTERISTICS OF *SALVIA CANDIDISSIMA* VAHL. SSP. *CANDIDISSIMA* (LAMIACEAE) AS A NEW RECORD FROM IRAN

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The present study, reports *Salvia candidissima* Vahl ssp. *candidissima* (Lamiaceae) as a new record for the flora of Iran. Anatomical and morphological characteristics of the plant have been investigated. Mainly based on the indumentum, corolla color and leaf morphology, *S. candidissima* is divided into two taxa including subsp. *candidissima* (leaves discolorous, ovate to broadly ovate; corolla bicolor) and subsp. *occidentalis* Hedge. (leaves concolorous, oblong to ovate; corolla homochromatic). Anatomical results show the general properties of the genus such as: quadrangular stem with thickened collenchyma at the corners and presence of sclerenchyma and collenchyma as mechanical tissue. Both glandular and eglandular trichomes on the stem and leaves are present. 2, 3-cellular eglandular and short capitate glands are the most common trichome types. An up-to-date distribution map of the species is provided. Moreover, the morphological description is amplified and diagnostic features are illustrated.

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**Key words:** New record; *Salvia*; Lamiaceae; anatomy; Iran

ویژگیهای تشریحی و ریخت شناسی *Salvia candidissima* Vahl. ssp. *candidissima* (تیره نعنا)، بعنوان گزارشی جدید برای فلور ایران

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در این نوشتار *Salvia candidissima* Vahl ssp. *candidissima* (نعنائیان) بعنوان گونه‌ای جدید برای فلور ایران گزارش می‌شود. ویژگی‌های تشریحی و ریخت‌شناسی گیاه مطالعه شده‌است. این گونه عمدتاً براساس صفات ریختی برگ و رنگ جام‌گل به دو زیرگونه subsp. *candidissima* (دارای برگ‌های تخم‌مرغی تا تخم‌مرغی پهن و جام‌گل دورنگ) و subsp. *occidentalis* Hedge (دارای برگ‌های کشیده تا تخم‌مرغی و جام‌گل تک‌رنگ) تقسیم می‌شود. از نظر تشریحی، صفات عمومی جنس از جمله ساقه چهارگوش در برش عرضی، کلانشیم توسعه یافته در زاویه‌های ساقه و حضور کلانشیم و اسکلراتشیم به عنوان بافت استحکامی در گیاه مشاهده می‌شود. هر دو نوع کرک غده‌ای و ساده در پوشش کرکی حضور دارند، درحالی‌که کرک‌های دو تا سه سلولی ساده و غده‌ای کوتاه فراوانترین نوع کرک هستند. نقشه پراکنش جدید گیاه تهیه شده است. بعلاوه، شرح گیاه‌شناسی کامل گونه ارائه شده است. در پایان، تصویر گیاه به همراه صفات ریخت‌شناسی کلیدی طراحی شده‌اند.

### INTRODUCTION

The genus *Salvia* L. (Salviniaceae, Nepetoideae,

Lamiaceae) with around 1000 species worldwide is the second largest genus of the family after *Nepeta* L. in

Iran. Three regions including Central and South America (near 500 spp.) likewise Western and Eastern Asia (near 300 spp.) have been assumed as species radiation centers of the genus. *Salvia* represents in Iran by 63 annual and perennial species, of which 17 are endemic (Eiji & Salmaki 2016; Jamzad 2012; Walker & Sytsma 2007; Kharazian & Shiran 2008; Hedge 1982a; Ranjbar & Pakatchi 2008). Traditionally, some well-known species of *Salvia* are used largely in Iranian folk medicine. It has been affirmed that different *Salvia* species show cardiotoxic, neuroprotective, antimicrobial, antioxidant, anticholinesterase, anti-apoptotic, antiglycating, antitumor and antimalarial activities (Asadi & al. 2010; Farimani & al. 2015; Hatipoglu & al. 2016; Naghibi & al. 2005; Shaerzadeh & al. 2011; Sonboli & al. 2006; Yousefzadi & al. 2007; Zeighami Alamdary & al. 2012). Moreover, the genus is rich in bioactive flavonoids and terpenoids (Bahadori & al. 2015). Camphor, bornyl acetate and borneole have been reported as the major essential oil constituents of *S. candidissima* (Özler & al. 2007).

Several studies have shown that some anatomical properties such as the shape of stem, petiole and midrib, arrangement and structure of the vascular bundles, sclerenchyma and collenchyma as well as morphological features of hair coverage provide valuable characteristics which can be applied as useful taxonomic tools for identification of genera and species in Lamiaceae. An investigation on *Salvia* and recently resurrected genus *Pleudia*, highlighted that trichome characters differ even between two close species belonging to the same section, but are invariant among different populations of a defined species. Such attitude underlines the taxonomic usage importance of trichome morphology in the genus (Akçin & al. 2011; Celep & al. 2014; Eiji & Salmaki 2016; Metcalfe & Chalk 1972; Nejad Habib Vash & Hosseini 2011; Özdemir 2016; Polat & al. 2015).

In the present study, *S. candidissima* ssp. *candidissima* is reported from Urmia, West Azerbaijan province, Iran as a new species for the flora of Iran (fig. 1). Conservation status of *S. candidissima* as a widely distributed species according to IUCN Red List Criteria, has been evaluated both at the national and global scales as LC (Least-Concern) status (Kahraman & al. 2012). Fortunately, by the present report of *S. candidissima* from Iran, the accounted conservation level is reinforced. Distribution map of the plant including the present locality is provided (map 1). Anatomical properties of the stem, petiole, leaf midrib and various trichome types are described. Also, the

light micrographs of the studied parts are presented. Finally, regarding to the discriminative significance of structural characteristics of the leaf, calyx, corolla and stamens in *Salvia* (Hedge 1982 a & b; Kharazian 2012), an illustration of the plant showing the foresaid diagnostic features is illustrated.

## MATERIALS AND METHODS

Plant specimens were collected from their natural mountainous habitat around Marmisho Lake located in Urmia, West Azerbaijan province of Iran in July 2012. Voucher specimens were deposited in the herbaria of the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University (MPH-1755), Tehran and Urmia Pharmacy School (HUPS-486), Urmia, Iran. Anatomical studies were done on the cross-sections of the stem, petiole and leaf midrib. Studied specimens were fixed in an Ethanol-Glycerol fixator (30: 70), were hand sectioned and stained with carmine and methylene blue. Thereinafter, stained samples were photographed by Olympus BX51 and Motic SMZ-168 light microscopes. Illustrations were drawn from the herbarium specimens by Mr. Sh. Bahadori.

## RESULTS

### *Salvia candidissima* Vahl. ssp. *candidissima*

Rhizomatous, aromatic perennial herb. Stems 25-70 cm, erect, quadrangle, ligneous at the base, lanate below, covered with short simple hairs, many capitate or sessile glands and some arachnoid hairs above. Leaves simple, ovate to broadly ovate, 5-16 × 3-12 cm, discolorous, pubescent to densely pannose, younger leaves albo-pannose, margins bi-serrate to erose, attenuate to cordate at base; petiole 3-7 cm. Inflorescence paniculate, yellowish-green, verticillasters 2-6 flowered, distant. Bracts broadly ovate-acuminate. Pedicels 2-4 mm. Calyx campanulate-tubular, 12-14 mm long, expanded in fruit, pilose to glandular-papillose, 13-veined, upper lip 3-dentated with bowed teeth, very close to each other, middle tooth smaller, lower lip 2-dentated with straight acuminate teeth. Corolla white, the middle lobe of the lower lip yellow, concave and slightly cut into two parts, lateral lobes oblong, acuminate, 22-28 (-30) mm, upper lip slightly two-lobed, strongly falcate with sparse sessile glands, tube 12 mm, ventricose, squamulate. Stamens exerted, with dolabriform sterile lower theca, connective long, 25-27 mm, filament short, 4 mm, adherent. Pistil bifid at the tip, 38-40 mm, with sparse short hairs. Nutlets rounded trigonus, 3-3.2 × 2.4-2.5 mm, smooth, darkly veined light brown (fig. 2).

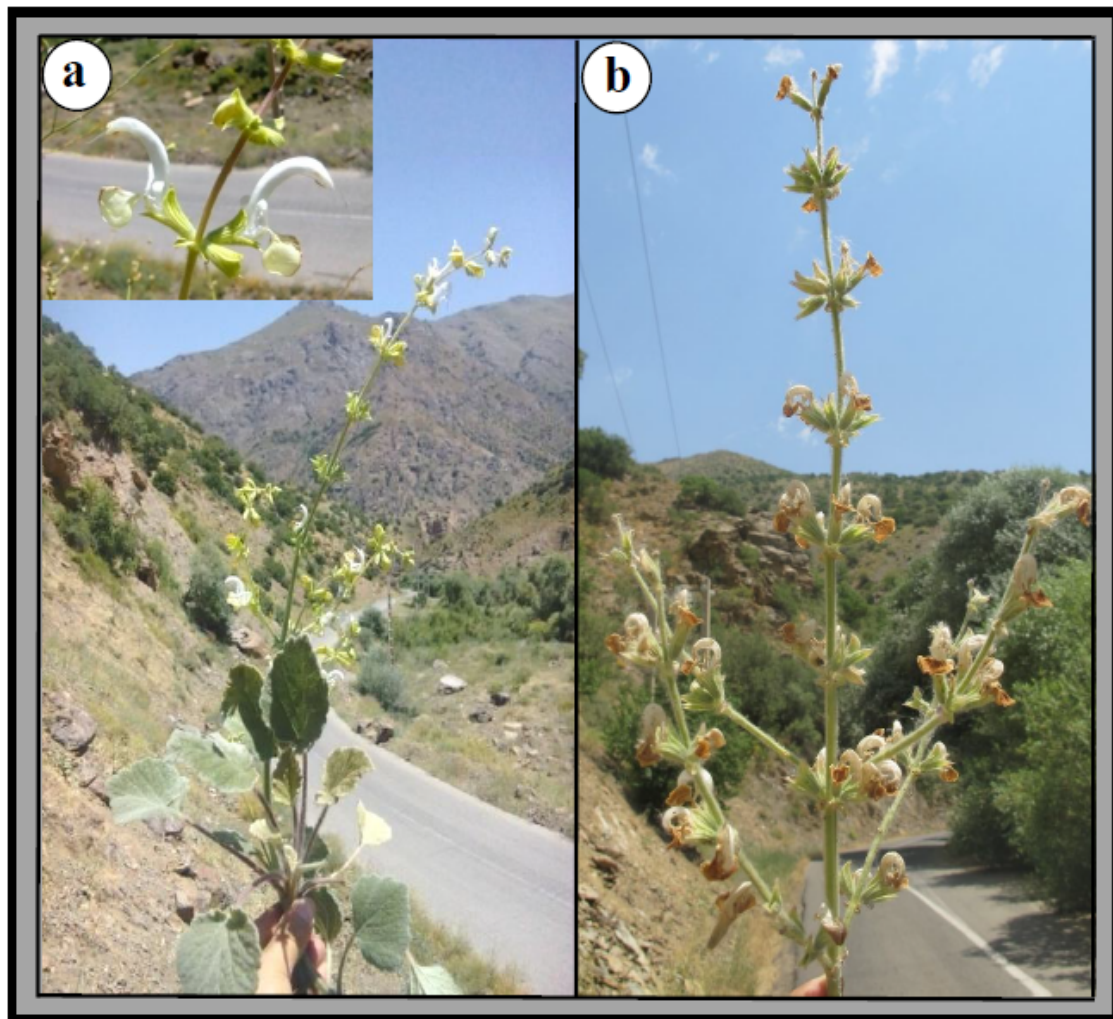
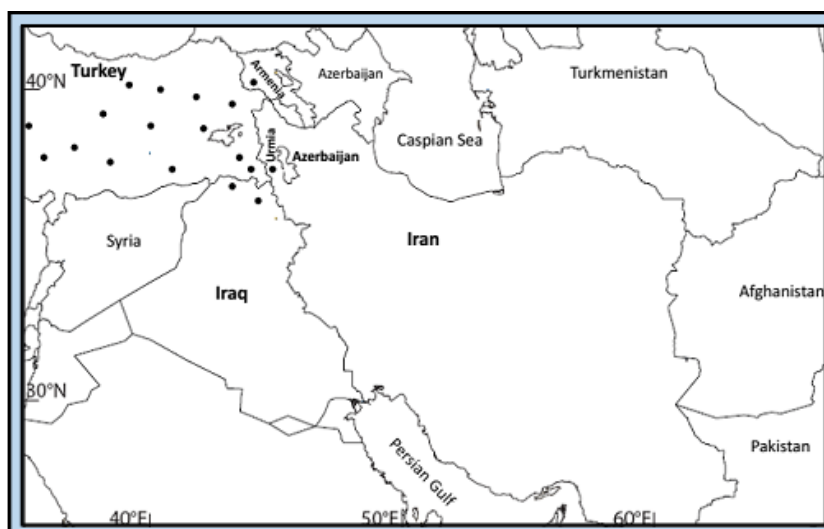


Fig. 1. *Salvia candidissima* ssp. *candidissima*. a, in the natural habitat and close up of flower; b, close up of the inflorescence. The photo was taken near Marmisho Lake, Urmia, West Azerbaijan, Iran.



Map 1. Geographical distribution of *Salvia candidissima* ssp. *candidissima*.

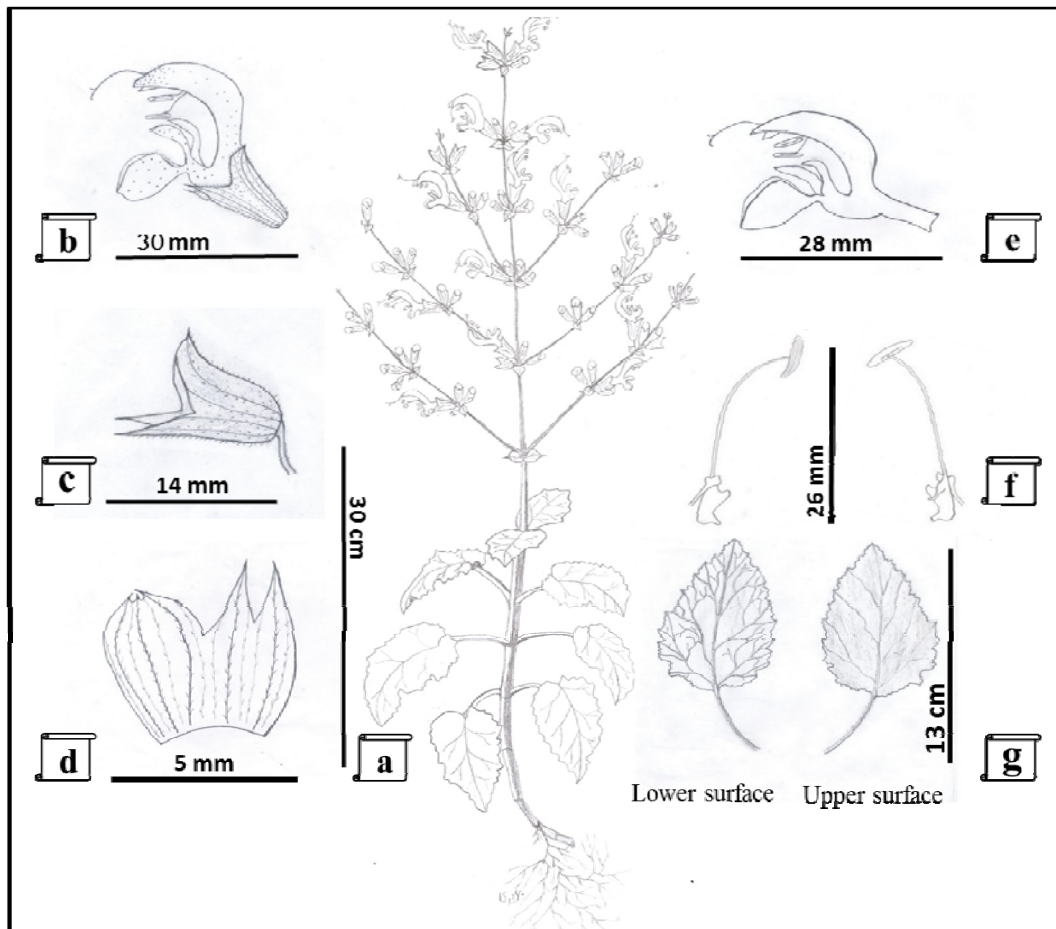


Fig. 2. *Salvia candidissima* ssp. *candidissima*. a, habit; b, flower; c, calyx; d, opened calyx; e, corolla; f, stamens; g, leaves.

## Anatomical characteristics

### Stem

Stem exhibits an obviously quadrangular configuration in the cross-section (fig. 3: 1-4). Epidermis is one-layered consisting of usually cubic cells, external cell walls protected by a thick (around 15-20  $\mu\text{m}$ ) cuticle layer. Indumentum is diverse and almost dense. Variable eglandular and glandular trichomes can be observed. Eglandular trichomes consist of straight or curved unicellular to multicellular hairs, 2 and 3-celled eglandular trichomes both are the most usual hair types on the stem. Unicellular hairs are rare. Glandular trichomes are peltate or capitate. Peltate trichomes are consisted of a basal cell, a short stalk and a multicellular secretory head. Capitate trichomes are composed of a basal cell, a uni to multicellular stalk, a

neck cell and usually a unicellular secretory head. Based on the size, capitate trichomes are long (length  $>100 \mu\text{m}$ ) or short (length  $<100 \mu\text{m}$ ). Short capitate trichomes are the most usual glandular trichome type on the stem (fig. 4). Multilayered collenchyma (about 8 layers) located under the epidermis is present at the stem corners. 2-3 layered chlorenchyma are found under the epidermis between the corners. The cortex parenchyma is 6-8 layered consisted of orbicular-hexagonal cells. Vascular bundles of the corners separated by parenchyma are obviously larger than the other ones. Phloem is located under the sheaths of sclerenchyma fibers. Cambium is not clear. Sclerenchyma protects xylem as well as phloem. Stem center is occupied by a large pith consisted of orbicular-hexagonal parenchymatic cells.

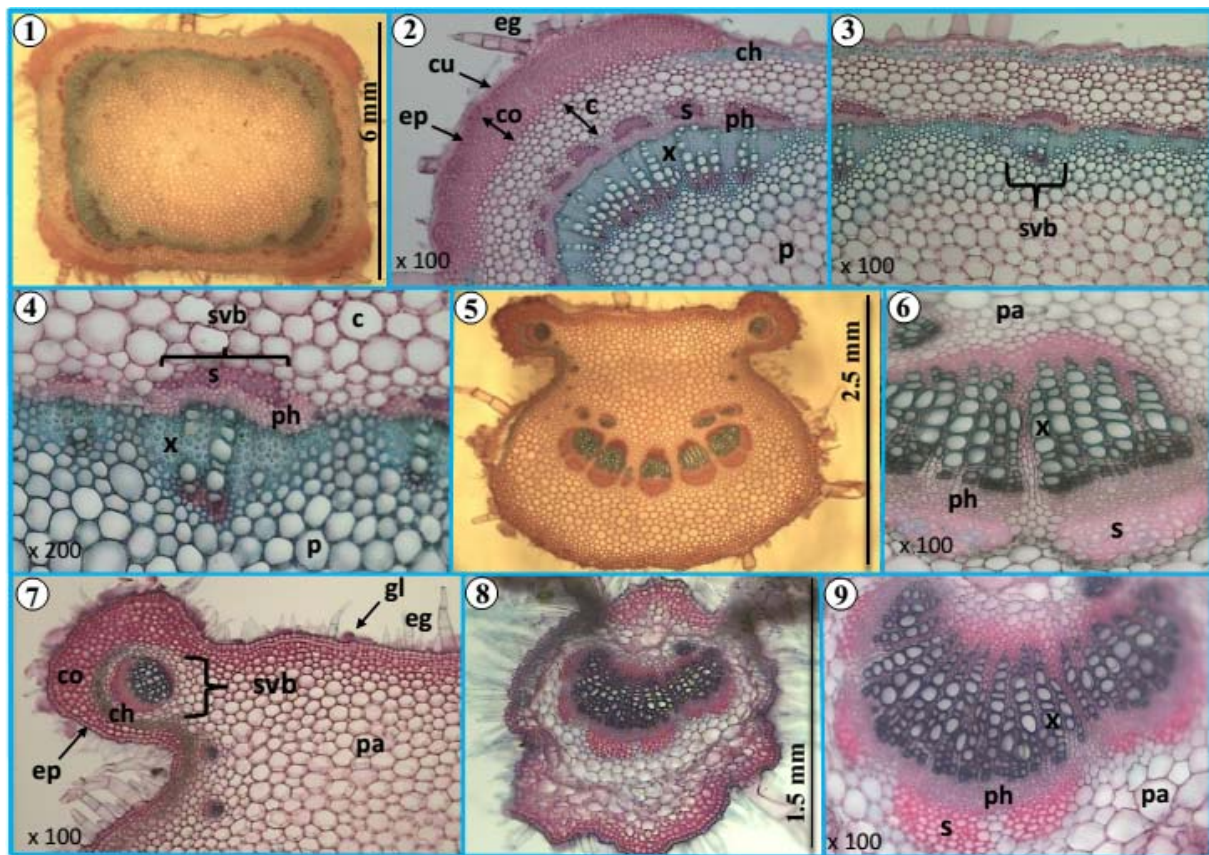


Fig. 3. Anatomical features of *Salvia candidissima* ssp. *candidissima*. 1-4, cross-sections of the stem; 5-7, petiole and 8-9, leaf midrib. 1, stem, complete cross-section ; 2, close up of the corner; 3-4, subsidiary vascular bundles; 5, complete cross-section of petiole; 6, close up of the central vascular bundles; 7, the crest; 8, leaf midrib, complete cross-section ; 9, close up of the vascular bundles. c: cortex; ch: chlorenchyma; co: collenchyma; cu: cuticle; eg: eglandular trichome; ep: epidermis; gl: glandular trichome; p: pith; pa: parenchyma; ph: phloem; s: sclerenchyma; svb: subsidiary vascular bundles; x: xylem.

### Petiole

The petiole is circular in the cross-section with two crests, the adaxial surface is almost flat and the abaxial one is strongly convex. Epidermis is one-layered, consisted of cubic to oval cells, covered with an almost thick (about 7-10  $\mu\text{m}$ ) cuticle layer (fig. 3: 5-7). Indumentum is diverse and dense. Eglandular trichomes show different shapes, straight or curved, usually 2, 3 or 4-celled. The most common hair types on the petiole are 2 and 3-celled eglandular trichomes. Unicellular eglandular trichomes are rare. Comparatively the petiole has fewer glandular trichomes than the stem, including short and long capitate and a few peltate glandular trichomes (fig. 4). Short capitate glands are the most usual glandular trichome type. Collenchyma located under the epidermis is thicker at the both adaxial and abaxial surfaces than lateral sides, well expanded (up to 6 layers) around the outer parts of subsidiary vascular

bundles situated in the petiole crests. 3-4 layered chlorenchyma is found under collenchyma in the crests. Inner parts of the petiole are occupied with orbicular-hexagonal parenchymatic cells. Collaterally arranged central vascular bundles are of divided type consisted of 10 smaller, distinct and polymorph vascular bundles separated by parenchymatic cells. Phloem is located under almost thick sheets of sclerenchyma. Cambium is not clear. Xylem is also protected by sclerenchyma. Moreover, about 6 rounded smaller vascular bundles can be found along the lateral walls of petiole between the central vascular bundles and crests.

### Leaf midrib

Both adaxial and abaxial surfaces of the median vein of leaf are convex, the abaxial surface strongly cambers around the midrib. The midrib is encircled with a single-layered epidermis consisted of oval to cubic cells, cells of the lower epidermis is smaller than

those of the upper layer. The epidermis is covered with a dens glandular and eglandular indumentum. Collenchyma located under the epidermis is clearly thicker at both adaxial and abaxial surfaces than other

parts. Parenchyma consisted of large irregular cells. A large vascular bundles surrounded by 2-6 layered sclerenchyma is present in the center of midrib and more or less appears as a crescent (fig. 3: 8-9).

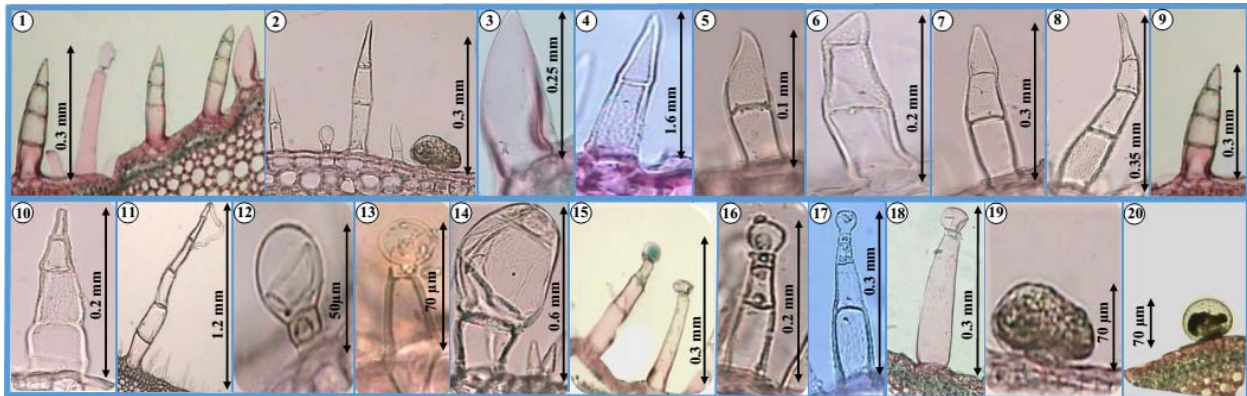


Fig. 4. Different trichome types of *Salvia candidissima* ssp. *candidissima*. 1-2, general indumentum perspectives; 3-11, unicellular to multicellular eglandular hairs; 12-18, capitate and 19-20, peltate glandular trichomes.

## DISCUSSION

In the present study *S. candidissima* ssp. *candidissima* is reported as a new record of the Lamiaceae family for Iran, from West Azerbaijan province, Urmia. It is an aromatic sturdy plant with hemicryptophyte life-form which prefers mountainous (1500-1800 m) habitats in the Irano-Turanian Region (Hedge 1982b).

In our area, *S. candidissima* grows on the rocky limestone slopes of Marmisho woodlands sharing its habitation with *Michauxia laevigata* Vent., *Achillea filipendulina* Lam., *A. vermicularis* Trin., *Marrubium astracanicum* Jacq., *Lamium garganicum* L., *Plumbago europaea* L., *Echinops ritrodes* Bunge, *Stachys iberica* M. Bieb., *Nepeta fissa* Benth., *Caucalis platycarpus* L., *Daphne mucronata* Royle, *Cratagus* sp., *Amygdalus* sp., *Astragalus* sp., *Celtis australis* ssp. *caucasica* (Willd.) C. C. Towns., *Pistacia atlantica* Desf. etc.

*S. candidissima* based on the leaf shape, corolla color and indumentum type is divided into two taxa: subsp. *candidissima* and subsp. *occidentalis*. The nominal subspecies as an Irano-Turanian element have its type from Armenia and also reported from northern Iraq and eastern parts of Turkey, while subsp. *occidentalis* is dominant in the western Turkey, Greece and Albania (Hedge 1982 a & b). The subspecies differ from each other by some diagnostic features e. g. cordate based ovate to broadly ovate and discoloured leaves with bi-serrate to erose margin in the eastern taxon and rounded to subcordate based oblong to ovate and concolorous leaves with serrulate to subentire

margin in the western taxon. Furthermore, flowers of subsp. *occidentalis* are partly smaller and quite white. But, the type taxon has larger, bicolor flowers (white and yellow). Specimens collected from Iran show extreme morphological features, quite distinct and easily recognizable from the subsp. *occidentalis*.

*S. candidissima* ssp. *candidissima* shows the general anatomical features of the genus. Stem is quite quadrangle strengthened by bulky collenchyma occurred in the corners. Petiole in the cross-section has a unique shape, almost circular with two rounded wings, which the adaxial surface is flat and the abaxial one is convex. Such characteristic can be used for determination of *Salvia* species (Nejad Habib Vash & Hosseini 2011; Özdemir 2016). The midrib either abaxially or adaxially is convex, with an irregular shape. In the genus *Salvia* midrib is surrounded by collenchyma (Metcalf & Chalk 1972) a characteristic that is obviously seen in the studied species.

Trichomes supply valuable morphological properties practicable in classification both at the inter- and infraspecific taxonomic levels of *Salvia*. Variable eglandular and glandular trichomes have been observed in different *Salvia* species (Bercu & al. 2011; Coisin & Gostin 2011; Eiji & Salmaki 2016). In the present species, 2 and 3-celled eglandular hairs both were the most usual simple trichome types. Unicellular eglandular trichomes were scarcely observed. Short capitate glands were the most common glandular trichome types. These observations are similar to the results of a recently performed study on 46 species of

the genera *Salvia* and *Pleudia* (Eiji & Salmaki 2016) which showed that the most usual trichome types on the studied species were short eglandular and short stalked glandular trichomes.

By the present species, the number of *Salvia* species known in Iran reached to 64 and the genus endemism rate is about 27 %. Obviously the endemic and narrowly distributed species are the most vulnerable members to extinction. Fortunately, *S. candidissima* is not an endangered species (Kahraman & al. 2012). But about Iran, during a field population census about 50 flowering individuals were identified by the authors distributed only in a restricted area. We believe that this species should be protected in Iran because of its small population size.

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